



The Effect of Elevated Levels of Thromboxane on Host Response to Tumor

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Previous studies have demonstrated that human malignancies can synthesize large amounts of thromboxane. It has also been reported that thromboxane can significantly alter multiple components of physiologic and immunologic function. We investigated the effect of elevated levels of thromboxane on host response to tumor using multiple rat models, and the long acting thromboxane analogue U-46619. Administration of the thromboxane analogue was not found to significantly alter the growth of primary tumors or peritoneal metastases. The analogue was found to significantly decrease mean survival time with a pulmonary metastases model. The thromboxane analogue failed to alter macrophage cytotoxicity, lymphocyte cytotoxicity, T lymphocyte subset numbers, or lymphocyte blastogenic response. Administration of the thromboxane analogue decreased the rate of lymphocyte metabolism of glucose and decreased lymphocyte intracellular adenosine deaminase activity. In conclusion, elevated thromboxane levels do not appear to alter primary tumor growth or host immune function, but do decrease resistance to pulmonary metastases.

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INTRODUCTION

Malignant human neoplasms have been demonstrated to exhibit altered rates of synthesis of the arachidonic metabolites [1]. Included in these alterations are an increased rate of synthesis of thromboxane by ovarian and breast carcinomas [2-4]. This finding is not universal in that malignant pulmonary tumors have failed to demonstrate altered rates of synthesis of thromboxane [5].

Alterations in the rate of synthesis of thromboxane may have clinical significance. Thromboxane has been demonstrated to exert multiple potent physiologic and immunologic effects [6]. These include an enhancement of immune function and a decrease in tissue perfusion [7,8]. In addition, pharmacologic inhibition of thromboxane synthesis has been reported to decrease the rate of tumor growth in experimental animal models [9]. The objective of our present study was to evaluate the effect

of elevated levels of thromboxane on the rate of growth of both primary and metastatic tumors and on the host response to these tumors through the use of multiple animal models.

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The care of all animals was in accordance with the guidelines set forth by the Animal Welfare Act and other federal statutes and regulations relating to animals in cities involved with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

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MATERIALS AND METHODS

Two different tumor cell lines were used to investigate the effect of elevated levels of thromboxane on tumor growth and host response. For the first, adult male Fischer rats weighing approximately 250 g were used as fibrosarcoma recipients. The animals were housed in individual stainless steel hanging cages and were allowed food and water ad libitum throughout the study. The animals were observed for a period of 1 week prior to entry into the study to exclude the presence of any pre-existing diseases.

The rats were implanted with 50 mg of a methylcholanthrene-induced fibrosarcoma using the technique previously described by Chance et al. [10]. Briefly, the animals were anesthetized by intraperitoneal sodium pentobarbitol (30 mg/kg). The backs were clipped of all hair and sterilized with an iodophor solution. A subcutaneous tunnel was surgically created dorsal to the spine commencing at the region of the lumbar vertebrae and extending to the region of the scapula. A 50 mg sample of the fibrosarcoma was aseptically obtained from a separate Fischer rat that had previously had the tumor implanted and allowed to grow to a size of approximately 50 g. This tumor aliquot was inserted through the incision to the distal most portion of the tunnel at the level of the scapula. The surgical incision at the base of the tunnel was closed with metal clips. The animals were returned to their cages and allowed to awaken. Beginning on the 28th day following tumor implantation the animals were randomly divided into two groups. One group received twice daily injections of 50 μ g/kg of a long acting thromboxane analogue U-46619. The second group received twice daily injections of lactated Ringer's solution and served as the control group. These agents were administered twice daily for the duration of the study. Tumor size was determined weekly using the technique of Chance et al. [10]. Briefly, the cross sectional dimensions of the tumor were determined in two planes using an engineers' caliper. These dimensions were used in the following formula to calculate tumor weight: length (mm) \times width (mm) \times 0.019 = tumor weight in grams. The animals were also weighed on a weekly basis commencing with the onset of drug therapy. A record was kept of the date of death of each animal to allow for comparison of mean survival time between groups. Following the death of each animal, the tumor was excised and weighed to determine final tumor weight. Tumor free carcass weights were obtained on all rats at the time of death.

Thirty Fischer rats underwent the same tumor implantation process and were begun on twice daily injections of lactated Ringer's solution ($n = 15$) or 50 μ g/kg of U-46619 ($n = 15$) commencing on post-tumor implantation day 28. These animals had cell mediated immunity assayed as previously described after 4 days of drug treat-

ment [11]. Briefly, the animals had the abdomens painted with 1 ml of a 0.5% dinitrofluorobenzene solution (DNFB) in a 4:1 volumes ratio of acetone: olive oil on the day of tumor implantation. On the third day after beginning treatment with lactated Ringer's solution or the thromboxane analogue, ear thickness of each rat was determined using an engineers' caliper. The ear was then painted with 0.1 ml of the 0.5% DNFB solution. Twenty-four hours later the animals were anesthetized with sodium pentobarbitol (30 mg/kg) intraperitoneally. Ear thickness was again measured and the percent ear swelling determined. Ear swelling as measured by this technique has previously been shown to correlate with cell mediated immunity [12]. The ears were amputated, fixed in formalin and stained with hematoxylin-eosin, and then assayed for leukocyte infiltration in response to the DNFB. A peripheral biopsy of the tumor was also obtained at this time and stained with hematoxylin-eosin. These biopsy specimens were also assayed for a leukocyte infiltration.

Leukocyte infiltration was quantified by enumeration of white blood cells in the tumor periphery or in the dermis of the DNFB treated ear. There were 15 high power fields (HPF) measured with each specimen using an image analysis system (Optomax, Hollis, New Hampshire) as previously described [13]. An average was obtained for these 15 fields.

Twenty additional Fischer rats were implanted with the same fibrosarcoma using the same surgical technique. Twenty-eight days following tumor implantation the rats began receiving twice daily intraperitoneal injections of lactated Ringer's solution ($n = 10$) or 50 μ g/kg of U-46619 ($n = 10$). After 4 days of therapy, the animals were anesthetized with intraperitoneal sodium pentobarbitol (30 mg/kg). The animals underwent celiotomy and had their spleens removed. The lymphocytes were purified using Ficoll-Hypaque centrifugation. The lymphocyte preparation so obtained was stained with anti-lymphocyte monoclonal preparations, washed, and reacted with affinity purified fluorescent-labelled goat anti-mouse IgG as a second step reagent. Fluorescent-labelled cells were then analyzed using standard flow cytometry. Cells were assayed for the presence of OX-19 (Pan T cells), W3/25 (helper/inducer T cells), and OX-8 (suppressor/cytotoxic T cells) membrane antigens.

For our second tumor cell line adult male Wistar-Furth rats weighing approximately 250 g were used in multiple colon cancer models. The tumor was obtained from a 1,2-dimethyl-hydrazine-induced, poorly differentiated syngeneic colon carcinoma. The tumor cell suspension utilized in these experiments was obtained by harvesting a tumor from a subcutaneous implantation site which has been allowed to grow to approximately 50 g in size. The tumor was mechanically disaggregated by slicing it into 1 mm³ pieces and vigorously shaking the suspension in

complete RPMI-1640 media. The cells were washed three times in the same media and the number of viable tumor cells determined using trypan blue staining. The cells were resuspended in sufficient RPMI media to achieve a final concentration of 1×10^6 cells/ml.

Three tumor models were used to evaluate the effect of elevated thromboxane levels on host response to this colon cancer cell line. In the first model (primary tumor model), 40 rats (control = 20; U-46619 = 20) had 1 ml of the tumor cell suspension (1×10^6 tumor cells) injected subcutaneously between the scapula. The animals received their initial dose of either lactated Ringer's solution or U-46619 immediately prior to being challenged with the tumor cell suspension. The animals were followed to death and mean survival times were determined. This model has previously been demonstrated to have a 100% mortality rate. The second model (peritoneal carcinomatosis model) involved the injection of 1×10^6 tumor cells intraperitoneally immediately following administration of the initial dose of either lactated Ringer's solution ($n = 20$) or U-46619 ($n = 20$). The animals were followed to mortality and mean survival time plus absolute survival rates determined. For the third model (pulmonary metastases model), the animals were injected with 1×10^6 of the tumor cells intravenously through the dorsal penile vein. Their first dose of either lactated Ringer's solution ($n = 20$) or U-46619 ($n = 20$) was administered immediately following tumor challenge. The rats were then followed to mortality and mean survival times were determined. This model has previously been demonstrated to have a 100% mortality rate.

To evaluate further the effect of elevated levels of thromboxane on host immunologic function, nontumor bearing adult Wistar-Furth rats were administered a 1 week course of either 50 $\mu\text{g/kg}$ of U-46619 or lactated Ringer's solution twice daily for 7 days. At the completion of the 7 day period, the animals were sacrificed by decapitation. The spleens were aseptically removed and homogenized in RPMI-1640. Lymphocytes were purified by density gradient centrifugation using Ficoll-Hypaque. The number of viable lymphocytes in each sample was determined and appropriate dilutions performed to achieve the desired concentration of lymphocytes.

Natural killer (NK) cell function of the lymphocyte preparations was determined using a 100:1 effector:target cell ratio. The assay was performed in a volume of 0.2 ml per well of RPMI media containing 1×10^6 lymphocytes and 1×10^4 YAC target cells which had previously been labelled with sodium ^{51}Cr chromate. After centrifugation at 40g for 2 minutes the plates were incubated at 37°C in 5% CO_2 for 4 hours. The plates were then centrifuged at 500g for 5 minutes and a 100 μl aliquot of the supernatant collected and assayed for ^{51}Cr on a gamma counter. The percentage of cell lysis was calculated as mean counts per minute released in the presence of effector cells minus

mean counts per minute spontaneously released by target cells incubated with media alone divided by the counts per minute after treating the cells with detergent minus the counts per minute spontaneously released with media alone; the quotient was multiplied by a hundred.

The lymphocyte preparations were also resuspended in sufficient RPMI media to achieve a final concentration of 5×10^6 cells per 0.2 ml of RPMI media. These suspensions were cultured in round bottomed microtiter plates for 72 hours in the presence of concanavalin A (Con A) or media alone for 72 hours. One microcurie of tritiated thymidine was added and the incubation continued for an additional 16 hours. The cells were harvested and tritiated thymidine incorporation was measured on a beta scintillation counter.

Nucleic acid metabolism was studied by incubating 1×10^7 Wistar-Furth splenic lymphocytes in 1 ml of complete RPMI media in flat bottomed culture plates with and without Con A stimulation in 5% CO_2 37°C for 48 hours. The wells were then aspirated and the aspirate frozen at -70°C and thawed at room temperature three times. The resulting cell lysates were assayed for adenosine deaminase activity and nucleotide triphosphate (NTP) levels. Adenosine deaminase activity was assayed using a colorimetric method described by Giusti [14]. Briefly, 0.05 ml aliquots of the thawed lymphocyte lysate were added to 1 ml of buffered adenosine solution. After incubation for 60 minutes at 37°C, 3 ml of phenol-nitroprusside solution and 3 ml of alkaline hypochlorite were added. After incubation for 30 minutes at 37°C absorbance at 628 nm (E) was measured. Volume activity was determined as follows:

$$\frac{(\text{E sample} - \text{E sample blank})}{(\text{E standard} - \text{E reagent blank})}$$

NTP was measured using a coupled enzymatic reaction [15]: $\text{NTP} + 3\text{-phosphoglycerate} \xrightarrow{\text{nucleotide diphosphate (NDP) + 1,3-diphosphoglycerate [1]}} \text{1,3-diphosphoglycerate} + \text{NADH} \rightarrow \text{glyceraldehyde 3-phosphate} + \text{NAD} + \text{phosphate [2]}$. Reaction 1 was catalyzed by phosphoglycerate kinase and reaction 2 was catalyzed by glyceraldehyde phosphate dehydrogenase. The change in absorbance at 340 nm which occurs when NADH is oxidized to NAD was measured.

The rate of glucose metabolism was determined as follows. Lymphocytes were cultured at a concentration of 1×10^7 lymphocytes/ml of RPMI media with and without Con A stimulation as described in the preceding paragraphs. After 6, 18, and 48 hours of incubation, aspirates were obtained of the culture media and analyzed for glucose concentration.

Elicited peritoneal macrophages were obtained by intraperitoneal administration of 4 ml of brain/heart infusate 4 days prior to sacrifice. Following 7 days of lactated

TABLE I. Tumor and Body Weights for Fibrosarcoma Bearing Fischer Rats From the Day of Initiation of Therapy With Lactated Ringer's Solution or the Thromboxane Analogue U-46619

Treatment day	Tumor weight (g)			Animal weight (g)		
	Lactated Ringer's solution	TBX ^a	<i>P</i> value	Lactated Ringer's solution	TBX	<i>P</i> value
0	35.9 ± 3.2	34.9 ± 2.7	0.815	280.0 ± 7.1	277.2 ± 3.3	0.723
6	50.3 ± 4.4	53.0 ± 4.8	0.680	276.9 ± 10.9	274.1 ± 4.1	0.800
12	62.3 ± 6.5	66.2 ± 4.9	0.641	285.9 ± 7.6	269.9 ± 6.4	0.132

^aTBX, thromboxane analogue U-46619.

Ringer's solution ($n = 10$) treatment or U-46619 ($n = 10$) treatment, the animals were sacrificed by decapitation. Midline celiotomies were performed aseptically and the peritoneal cavity lavaged with Hank's balanced salt solution without calcium or magnesium and with 0.25 mM EDTA. The resulting suspensions were hypotonically lysed of contaminating red blood cells and washed three times in standard Hank's balanced salt solution. The numbers of macrophages present were determined with a hemocytometer and the macrophages were resuspended in sufficient RPMI-1640 media to achieve the desired concentration.

Macrophage cytotoxicity was assayed against YAC cells as follows. Briefly, 5×10^4 ^{51}Cr -labelled YAC cells were mixed with 5×10^5 macrophages in a total volume of 0.2 ml RPMI media. The wells were cultured at 37°C and 5% CO_2 for 24 hours. The wells were then centrifuged and the supernatants from each well harvested and assayed for ^{51}Cr release. Cytotoxicity was calculated using the same formula as for the NK cell cytotoxicity assay.

Statistical Analysis

All data are expressed as mean \pm standard error of the mean. Comparisons between groups were made, using Fischer's exact test, the Wilcoxon test, and an unpaired *T* test. Significance was assumed at $P < 0.05$.

RESULTS

There was no significant effect of drug therapy on either tumor weight or animal weight using the subcutaneous fibrosarcoma model (Table I). At the time of animal death, the mean tumor weight in the control group was 72.1 ± 8.2 g and in the U-46619 treated group 83.4 ± 10.2 g. This difference was not statistically significant ($P = 0.402$). The tumor free carcass weight at the time of death in the control group was 161.5 ± 12.9 g and in the treatment group 153.2 ± 10.1 g. This difference was also not statistically significant ($P = 0.620$). The mean survival time for the control group was 18.2 ± 1.6 days following the onset of drug therapy

and for the thromboxane analogue treated group 15.7 ± 1.6 days. This difference was not significant ($P = 0.409$).

Treatment with the thromboxane analogue was not found to significantly alter cell mediated immunity as measured by ear swelling. The control animals have an average increase in ear thickness of $31.8 \pm 5.0\%$ and in the thromboxane analogue treated group $22.6 \pm 4.4\%$ ($P = 0.177$). Administration of the thromboxane analogue did significantly increase leukocyte infiltration into the hapten-sensitized ears. There were 64.5 ± 4.7 leukocytes/HPF in the control group and 109.6 ± 9.8 leukocytes/HPF in the U-46619 treated group ($P < 0.0001$).

Analysis of leukocyte infiltration of the fibrosarcoma revealed 88.8 ± 11.0 cells/HPF for the control group and 123.6 ± 15.5 cell/HPF for the thromboxane analogue group. This difference did not achieve statistical significance ($P = 0.076$).

Administration of the thromboxane analogue was not found to significantly alter the percentage of splenic *T* lymphocyte subsets. These data are shown in Table II.

The administration of a 1 week course of the thromboxane analogue to Wistar-Furth rats did not significantly alter NK cell cytotoxicity. Splenocytes harvested from the lactated Ringer's treated group lysed $12.76 \pm 1.06\%$ YAC cells, and from the U-46619 treated rats $10.68 \pm 0.74\%$ YAC cells ($P = 0.2152$). Administration of the thromboxane analogue also failed to alter macrophage cytotoxicity. Macrophages obtained from the control group lysed $31.56 \pm 4.03\%$ of the YAC cells and when obtained from the thromboxane analogue treated group $34.54 \pm 4.34\%$ ($P = 0.6211$).

The effect of the thromboxane analogue on survival in Wistar-Furth rats bearing the colon cancer cell line is shown in Table III. Administration of the thromboxane analogue was not found to significantly alter mean survival time with subcutaneous implantation of the tumor ($P = 0.2579$). The thromboxane analogue was also not found to alter mean survival time or absolute survival rate with the intraperitoneal carcinomatosis model ($P = 0.3849$ and 0.3422 , respectively). The thromboxane ana-

TABLE II. Percentage of T Lymphocyte Subset Populations for Fischer Rats Administered a 1 Week Course of Treatment of Lactated Ringer's Solution or the Thromboxane Analogue U-46619

	Lactated Ringer's solution	TBX ^a	P value
Pan T cell (OX-19)	68.4 ± 5.4%	76.2 ± 3.6%	0.256
Helper/inducer T cell (W3/25)	45.2 ± 2.0%	44.0 ± 1.6%	0.643
Suppressor/cytotoxic T cell (OX-8)	25.5 ± 1.7%	30.2 ± 2.3%	0.135

^aTBX, thromboxane analogue U-46619.**TABLE III. Survival Data for Wistar-Furth Rats Challenged With Multiple Colon Cancer Models and Administered the Thromboxane Analogue U-46619 or Lactated Ringer's Solution**

	Lactated Ringer's solution	TBX ^a	P value
Mean survival times for peritoneal carcinomatosis model (days)	44.35 ± 1.73	42.35 ± 1.68	0.3849
Absolute survival rates for peritoneal carcinomatosis model	60%	45%	0.3422
Mean survival times for pulmonary metastases model (days)	39.84 ± 1.85	33.22 ± 1.14	0.0009
Mean survival times for primary tumor model (days)	34.13 ± 0.75	35.43 ± 1.61	0.2579

^aTBX, thromboxane analogue U-46619.**TABLE IV. Effect of Administration of the Thromboxane Analogue U-46619 on Nucleic Acid Metabolism in Splenic Lymphocytes Obtained From Wistar-Furth Rats**

	Lactated Ringer's solution	TBX ^a	P value
Con A mitogen response (cpm)	155,708 ± 21,209	134,052 ± 16,218	0.4318
No Con A blastogenesis (cpm)	1,358 ± 112	1,718 ± 478	0.4842
Adenosine deaminase activity without Con A (units/10 ⁷ cells)	7.1 ± 0.5	4.60 ± 0.60	0.0055
Adenosine deaminase activity with Con A (units/10 ⁷ cells)	23.1 ± 2.4	11.30 ± 2.00	0.0010
NTP without Con A (ng/10 ⁷ cells)	848.1 ± 158.8	1,213.40 ± 166.40	0.1317
NTP with Con A (ng/10 ⁷ cells)	843.6 ± 84.8	859.30 ± 197.60	0.9428

^aTBX, thromboxane analogue U-46619.

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logue did significantly decrease mean survival time with the pulmonary metastases model ($P = 0.0009$).

The effects of the thromboxane analogue on nucleic acid metabolism are listed in Table IV. Neither spontaneous lymphocyte blastogenesis nor Con A-induced blastogenesis was significantly altered by administration of the thromboxane analogue ($P = 0.4842$ and 0.4318 , respectively). The thromboxane analogue was not able to alter intracellular NTP levels of either unstimulated or Con A stimulated lymphocytes ($P = 0.1317$ and 0.9428 , respectively). Adenosine deaminase activity was found to be diminished in both unstimulated and Con A stimulated

lymphocytes obtained from U-46619 treated rats ($P = 0.0055$ and 0.0010 , respectively).

Lymphocytes obtained from the thromboxane analogue treated rats were noted to have a decreased rate of glucose metabolism as measured by its rate of disappearance from the culture media (Table V).

DISCUSSION

It has been recognized for over a decade that malignant neoplasms can synthesize increased amounts of thromboxane. It has also been known that elevated levels of thromboxane can modify both primary tumor growth and



TABLE V. Glucose Concentration (mg/dl) in RPMI Media Containing 1×10^7 Splenic Lymphocytes/ml Obtained From Wistar-Furth Rats Treated With the Thromboxane Analogue U-46619 or Lactated Ringer's Solution

Incubation time (hours)	With Con A stimulation		
	Lactated Ringer's solution	TBX ^a	P value
6	200.2 \pm 0.6	194.9 \pm 0.9	0.0002
18	187.6 \pm 1.3	187.9 \pm 1.3	0.8728
48	152.4 \pm 5.0	175.3 \pm 5.1	0.0050

^aTBX, thromboxane analogue U-46619.

the host response to the tumor. Honn and Meyer demonstrated that addition of thromboxane to in vitro tumor cell cultures resulted in decreased cyclic adenosine monophosphate (AMP) levels and an increased rate of tumor proliferation [16]. In contrast, the administration of thromboxane synthetase inhibitors failed to alter the rate of metastasis of tumors in multiple mouse models [17]. This apparent discrepancy may be due to one or two factors. First, it is possible that the in vitro data obtained by Honn and Meyer do not correlate to in vivo situations. Second, the administration of thromboxane synthesis inhibitors has been demonstrated to result in increased rates of synthesis of other arachidonic acid metabolites which could in themselves alter either immune function or tumor growth [17]. The purpose of our current study was to eliminate these variables by administering a thromboxane analogue, rather than a thromboxane synthetase inhibitor, and to utilize multiple in vivo models.

The thromboxane analogue failed to alter the rate of growth of a fibrosarcoma in Fischer rats. The thromboxane analogue also failed to alter the rate of tumor growth with either the subcutaneous implantation model or the peritoneal carcinomatosis model utilizing the Wistar-Furth colon cancer cell line. Administration of the thromboxane analogue did decrease mean survival time with the pulmonary metastases model.

There would appear to be three possible mechanisms for the decreased mean survival time with the intravenous pulmonary metastases model. First, the analogue may have primarily altered the kinetics of the tumor cell growth. This however, would appear to be unlikely since such an effect would also have been expected to be present with both the subcutaneous and peritoneal carcinomatosis models of this tumor cell line.

A second possible explanation is that the thromboxane analogue altered the host's immune response to the tumor. We were however, unable to demonstrate any alteration in macrophage or lymphocyte cytotoxicity against tumor cells, lymphocyte blastogenic response, or T cell subset numbers. Rather, the thromboxane analogue merely decreased the intracellular adenosine deaminase activity, and the rate of glucose consumption by lympho-

cytes. Although such parameters are considered to be important for normal immune function [18,19], the decreases were apparently not sufficient to result in any immunosuppression as measured by the functional assays we performed. The thromboxane analogue did increase the rate of leukocyte infiltration of the hapten treated ears. This, however, would appear to be an immunostimulatory effect and as such would not be expected to impair a host resistance to the tumor.

The final possibility for the adverse effect in the pulmonary metastatic model is that the thromboxane analogue altered the host response to the tumor in a nonimmunologic manner. This could be the result of the fact that thromboxane stimulates platelet adherence [1]. Platelets so stimulated will adhere among other surfaces, to tumor cells. This process greatly increases the ability of such cells to adhere to endothelial cells, migrate between the cells, escape destruction by native leukocytes, and establish successful metastatic colonies [20]. Such an effect would be more important for blood borne metastases than for lymphatic metastases or peritoneal metastases. Our finding that the thromboxane analogue did not alter primary tumor growth or peritoneal metastatic tumor growth but did increase the rate of blood borne pulmonary metastases would thus tend to support this hypothesis. Further studies utilizing tagged platelets obviously will be required in order to yield a more definitive answer to this question.

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COMMENTS

The study by Waymack et al. [11] addresses an important question: What role do the eicosanoids play in tumor metastases? Of these biologically active molecules the

investigators chose to study thromboxane which has reported immunologic effects in addition to potent physiologic effects and has an increased synthesis rate in certain cancers. They have evaluated thromboxane's effect in immunoresponsiveness and different types of metastases: subcutaneous, peritoneal, and pulmonary. Their data show that the administration of thromboxane did not alter growth of the primary tumor or host immunity, as measured, and did affect pulmonary metastases only (significant decrease in survival time). The discussion in interpreting the results is excellent with their conclusion that to confirm the probable role of platelet aggregation secondary to the thromboxane, certain platelet experiments are in order. In addition to this possibility, the vasoconstrictive properties need investigation. For some reason in this model tumor nutrition from vascular stasis was not operative contrary to its usual role reported by others. One might wonder if the administered/circulating and long acting analogue acts similarly to the thromboxane synthesized/released from cell injury. At any rate, many of these and other questions can be answered by these tumor/animal models.

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